Assessment of *Escherichia coli* isolates for *In vitro* biofilm production

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Abstract

A total of 14 *Escherichia coli* isolates were assessed for their ability to produce biofilm in-vitro by slime production on Congo red agar medium (CRA) and microtitre plate assay. Out of 14 isolates tested, 12 were slime producing on CRA as indicated by black colonies. The isolates of *E.coli* varied in their ability to produce biofilm on the surface of microtitre plate ranging from 0.101 to 0.543 ODm. Out of 14 isolates tested, 10 were positive for biofilm production employing criterion of blank corrected ODs9s > 0.1. Two of slime negative isolated were also negative for biofilm production where as the two slime positive isolates were found to be negative for biofilm production.

Keywords: Biofilm, *E.coli*, Slime production, Microtitre plate assay, *In vitro.*

Introduction

Traditionally, bacteria have been primarily characterized as planktonic, freely suspended cells and described on the basis of their growth characteristics in nutritionally rich culture media. Recently, scientists have realized that in the natural world bacteria live in micro ecosystems filled with hundreds of other microorganisms i.e. biofilms. Biofilm mode of bacterial growth exhibits a distinct phenotype with respect to altered gene transcription and growth rate as well as increased resistance to chemical and physical treatments. Biofilms are defined as assemblage of microbial cells that are irreversibly associated with a surface and enclosed in a matrix primarily of polysaccharides. (Costerton et al., 1995).

The term biofilm and slime are often used interchangeably (Ammendolia et al., 1999). The biofilm formation is considered to be two-step process in which the bacteria first adhere to a surface mediated by capsular antigen or flagellar antigen,followed by multiplication to form a multilayered biofilm, which is associated with production of exopolysaccharide matrix. The ability of bacteria to form biofilms helps them to survive hostile conditions within host and is considered to be responsible for chronic or persistent infections. (Costerton et al., 1999). A mechanism of adherence to the intestinal epithelium has been suggested for ETEC in-vivo, where fimbriae are responsible for initial adhesion and Capsular exopolysaccharide are responsible for the formation of microcolonies in which bacteria multiply. (Chan et al., 1982).

Slime production and microtitre plate procedure are indirect methods for the assessment of bacteria for biofilm production in vitro and has been used with the modifications previously. (Cucarella et al., 2001, Djordjevic et al., 2002; Borucki et al., 2003, Vasudevan et al., 2003, Wakimoto et al., 2004).

The study was aimed to employ slime production on Congo red agar medium (CRA) and micro titre plate method for the assessment of in-vitro biofilm production of *Escherichia coli* isolates available in the Department.

Materials and Methods

Bacterial isolates:

A total of 14 *Escherichia coli* cultures available in the Department were used for the study.

Slime production assay:

Slime production in bacterial cultures was determined by cultivation on CRA (Congo Red Agar) plates. (Freeman et al., 1989). Inoculated plates were incubated at 37° C for 24 hr followed by storage at room temperature for 48 hr. The production of rough black colonies by bacterial cultures indicated the slime production.

Microtitre plate assay:

The method of Borucki et al., (2003) was followed to determine the ability of varIOus bacterial cultures to

produce biofilm in-vitro, with slight modifications.

Briefly, each culture was individually grown overnight in Trypticase Soya Broth (TSB) at 37°C, and diluted I : 40 in TSB containing 0.25% glucose. The sterile 96 well "U" bottom polystyrene tissue culture plate was inoculated with 200111 of bacterial cell suspension, and incubated for 24 he at 37 °C without agitation. The outer row and column of plate was kept uninnoculated but filled with sterile PBS to avoid dehydration of cells growing. The wells were washed thrice with sterile Phosphate I Buffer Saline (PBS, pH 7.4), dried at room temperature and finally stained with 0.1 % crystal violet solution for 30 minutes.

After rinsing thrice with sterile distilled water and subsequent drying, the stain taken up by the adherent biofilms was extracted with 200111 of 95 % ethanol for 30 min. at 4°C. The content of each well (100 Ill) was transferred to a sterile microtitre plate and the optical density at 595 nm (OD595) of each was measured in microtitre plate reader. The isolate with blank corrected OD>O.1 was considered as positive for biofilm production. Each culture was tested in triplicates and the assay was performed twice.

Results and Discussion

The biofilm production by microtitre plate assay has been performed for numerous bacteria including E.co/i. (Cucarella et al., 2001, Djordjevic et al., 2002; Borucki et al., 2003, Vasudevan et al., 2003, Wakimoto et a!., 2004). The microtitre plate has the advantage of being simple and can be easily modified to analyze the multiple strains or growth conditions within each experiment.

Out of 14 *E.coli* isolates tested, 12 were found to produce black colonies within 24-48 hr. The remaining 2 isolates failed to produce black colonies even after 72 hr. of incubation. The observation of slime production in *E.coli* seems to be novel and this can be taken up as a qualitative assessment of biofilm producing ability. Such slime production has been reported in other bacteria like Staphylococcus aureus (Ziebur et al, 1997; Aricola et al., 2001; Vasudevan et al., 2003). Slime production reflect the capacity of bacteria to adhere specific host tissues and there by to produce invasive microcolonies. (Baselga et al., 1994).

The isolates of *E.coli* varied in their ability to produce biofilm on the surface of microtitre plate ranging from 0.101 to 0.543 ODS9s. Out of 14 isolated tested, 10 were positive for biofilm production employing criterion of blank corrected OD > 0.1. Two of slime negative isolated were also negative for biofilm production where as the two slime positive isolates were found to be negative for biofilm production.

Such difference among strains of a bacterium has also been noted. Wakimoto et al., (2005) performed microtitre plate assay for the screening of Enteroaggregative *E.coli* and found that strains with >0.2 ODS70 were EAEe. Djordjevic et al. (2002) and Borucki et al. (2003) also noted variation within strains of Listeria monocytogenes to produce biofilm in-vitro. Based on such variation, Borucki et al.(2003) also differentiated strong and weak biofilm forming strains of L.monocytogenes. Vasudevan et al. (2003) also reported such variation in biofilm production by *Staphylococcus aureus* strains.

The failure of two slime positive isolates to produce biofilm in-vitro can be attributed to the variation in phenotypic expression of biofilm and / or slime production. Such observations were also made by Vasudevan et aI., 2003 where they found that out of 35 *S.aureus* isolates, 32 strains were slime positive and only 24 produced biofilm on microtitre plate. Three slime negative strains were also negative for biofilm production but one slime negative strain produced biofilm. The studies by Basgela et al., (1993); and Christensen et al., (1987) revealed that the phenotypic expression ofbiofilm formation is highly susceptible to in vitro conditions.

This study concludes with variation in slime and bio.film producing capacity of *E.coli* isolates by Slime production on CRA and biofilm production in microtitre plate.

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Table - 1 showing optical density values for crystal violet absorbance and slime production on CRA.

Culture No.	CV absorbance value			Slime production on CRA
	MeanOD595	Blank corrected Od595	Value	
E1	0.241	0.128	TRUE	+ve
E2	0.427	0.314	TRUE	+ve
E3	0.164	0.051	FALSE	-ve
E4	0.204	0.091	FALSE	-ve
E5	0.214	0.101	TRUE	+ve
E6	0.175	0.062	FALSE	+ve
E7	0.288	0.175	TRUE	+ve
E8	0.257	0.144	TRUE	+ve
E9	0.277	0.164	TRUE	+ve
EIO	0.245	0.132	TRUE	+ve
Ell	0.173	0.060	FALSE	+ve
E12	0.656	0.543	TRUE	+ve
E13	0.233	0.120	TRUE	+ve
E14	0.314	0.201	TRUE	+ve
Control	0.113	-----	----	----

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